SPECIFICATION AMENDMENTS

Page 16, sole paragraph, continuing onto page 17:

Fused Deposition Modeling (FDM) is an additive manufacturing process that forms 3D objects through the extrusion and deposition of individual layers of thermoplastic materials. It begins with the creation of a conceptual CAD model on the computer. The model is imported into software (e.g., the ~~QuickSlice~~ QUICKSLICE™ software offered by ~~Stratasys~~ STRATASYS Inc. of Eden Prairie, MN) which mathematically slices the conceptual model into horizontal layers. This is followed by the creation of deposition paths within each sliced layer. The tool path data is then downloaded to the FDM machine for scaffold fabrication. A software package (e.g., ~~SupportWorks~~ SUPPORTWORKS offered by ~~Stratasys~~ STRATASYS Inc.) automatically generates supports if needed. The FDM system operates in the X, Y and Z axes. In effect, it draws the designed model one layer at a time. The FDM method involves the melt extrusion of filament materials through a heated nozzle and deposition as thin solid layers on a platform. The nozzle is positioned on the surface of a build platform at the start of fabrication. It is part of the extruder head (FDM head), which also encloses a liquefier to melt the filament material fed through two counter-rotating rollers. Each layer is made of "raster roads" deposited in the x and y directions. A "fill gap" can be programmed between the roads to provide horizontal channels. Subsequent layers are deposited with the x-y direction of deposition - the "raster angle" - programmed to provide different lay-down patterns.

Page 30, last paragraph, continuing onto page 31:

Polycaprolactone (PCL) pellets can be obtained from ~~Aldrich~~ ALDRICH Chemical Company, Inc. (Milwaukee, Wisconsin) (Catalog No. 44,074-4). The semi-crystalline bioresorbable polymer has an average M_n of ca. 80,000 (GPC) with a melt index of 1.0 g/10 min (125°C/44 psi ASTM D1238-73). It has a melting point of 60°C (DSC) and a glass transition temperature of -60°C. The polymer pellets are kept in a dessicator prior to usage.

Page 31, full paragraph:

Filament fabrication is performed using a fiber-spinning machine (~~Alex James~~ ALEX JAMES & ~~Associates~~ ASSOCIATES Inc., Greensville, S.C.). PCL pellets are melted at 190°C in a cylinder by an external heating jacket. After a hold-time of 15 min, the temperature is lowered to 140°C and the polymer melt is extruded through spinnerets with a die exit diameter of 0.064" (1.63 mm). Each batch of PCL pellets weighs 30 ± 1 g. The piston speed is set at 10 mm/min. The extrudate is quenched in chilled water, placed 40 mm below the die exit. The combination of temperature, piston speed and height-drop to water quenching settings: produces a filament diameter of 1.70 ± 0.10 mm. The PCL filaments are fabricated to have a consistent diameter to fit the drive wheels of an unmodified FDM system. The filaments are vacuum-dried and kept in a dessicator prior to usage.

Page 33, last paragraph, continuing onto page 34:

Scaffolds are fabricated using PCL or PCL/HA filaments with a FDM 3D ~~Modeler~~ MODELER rapid prototyping system from ~~Stratasys~~ STRATASYS Inc. (Eden Prairie, MN). Slices of the CAD model in .STL format are generated on Stratasys' ~~QuickSlice~~ QUICKSLICE (QS) software with a specified slice thickness. The amount and direction of the extrudate (called "raster" in FDM's context) are determined by configuring various build parameters for individual layers. The build parameters include the road width of rasters, fill gap between rasters, slice thickness and raster angle. Specific combinations of these parameters and the liquefier temperature are required to achieve smooth and consistent extrudate flow and raster deposition with sufficient adhesion between adjacent layers. The head speed, fill gap, and raster angle for every layer are programmed through the QS software and saved as an .SLC, the (Slice) file format. The 2D slice data is then converted into the QS.SML (~~Stratasys~~ STRATASYS Inc. Machine Language) file format that automatically generates the build paths based on the input parameters for each slice layer. The FDM parameters for the processing of PCL and PCL/HA composite scaffolds are set out in Table 1. Table 2 shows the effect of these parameters on the geometry of the scaffold structure. The .SML data is sent to the FDM machine to fabricate the scaffold, specimens. The liquefier temperature is set at 120°C ± 10°C. and the envelope temperature remains at 25 ± 2°C (ambient temperature) throughout the fabrication process.

Page 36, first paragraph:

Software: ~~Stratasys' Quickslice~~ STRATASYS' QUICKSLICE (QS)
Material Dependant Values:

(workable range for PCL and PCL/HA filaments Ø1.8 ± 0.1 mm)

Page 48, last paragraph, continuing onto page 49:

Scaffold specimens were fabricated using PCL and PCL/HA filaments as described above with a FDM 3D ~~Modeler~~ MODELER rapid prototyping system from ~~Stratasys~~ STRATASYS Inc. (Eden Prairie, MN). Blocks of 32.0 (length) x 25.5 (width) x 13.5 mm (height) with a 61% porosity were created directly in the ~~Stratasys' QuickSlice~~ STRATASYS' QUICKSLICE (QS) software. The head speed, fill gap, and raster angle for every layer were programmed through the QS software and saved as an .SLC, the (Slice) file format. Lay-down patterns of 0/60/120° and 0/72/144/36/108° were used to give a honeycomb-like pattern of triangular and polygonal pores, respectively.

Page 49, full paragraph:

The 2D slice data were converted into QS's .SML (~~Stratasys~~ STRATASYS Machine Language) file format that automatically generated the build paths based on the input parameters for each slice layer. The .SML data was sent to the FDM machine to fabricate the scaffold specimens using a T16 tip. The liquefier temperature was set at 120°C and the envelope temperature remained at 25 ± 2°C (ambient temperature) throughout the fabrication process. After manufacture, the four specimens of each configuration were cut with an ultra sharp blade into slightly smaller blocks with dimensions 28.0 (length) x 21.0 (width) x 13.5 mm (height). One each of these new blocks was used to measure the porosity of the design and then all four

specimens were each divided into twelve small scaffold specimens (6.5 x 6.5 x 13.5 mm) for microscopically characterization and compression testing, as described in the following sections.

Page 56, paragraph continuing onto page 57:

Primary human osteoprogenitor cells were isolated under sterile conditions by stripping periosteum from the skull of a patient who underwent craniofacial surgery. Monolayer cell culture flasks were filled with 3 to 5 small pieces and Dulbecos Modified Eagle medium (~~Gibco~~ GIBCO, Grand Island NY, USA) with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin and 1% amphoterecin. In addition, 50.0 mgL ascorbic acid was added to promote the osteoblastic phenotype. Then the flasks were placed in a self-sterilizable incubator (WTB Binder, Tuttlingen, Germany) at 37°C in 5% CO_2, 95% air, and 99% relative humidity. After the monolayer-culture grew to confluence and exhibited nodule formation, the osteoblast-like cells were harvested using a 0.05% trypsinethylendiamine tetra-acetic acid (EDTA) solution, split, resuspended in culture medium and filled into new culture flasks. The culture media was replaced every third or fourth day. Alkaline phosphatase and osteocalcin staining verified the osteoblastic-phenotype. After confluent monolayers were achieved by the fourth passage, the cells were enzymatically lifted from the flask using 0.25% Trypsin/EDTA (~~Hyclone~~ HYCLONE Utah, USA) and counted using a hemocytometer. The cell mortality was less than 5% as shown by trypan blue staining and maintained a stable cell metabolism. Cell pellets were resuspended and aliquots of 15 µml containing 50,000 cells were seeded onto the top of PCL scaffolds measuring 6x6x2 mm. Subsequently, the seeded scaffolds

were placed into an incubator to allow the cells to build adhesion plaques on the polymer surface. After 2 hours, 1 ml of complete media was added into each well. Cell-scaffold constructs were then cultured for a period of 3 weeks.

Page 57, last paragraph continuing onto page 58:

The establishment of the fibroblast and osteoblast-like phenotype, intercellular connections, and extracellular matrix production were examined daily by phase-contrast light microscopy for three and four weeks, respectively. Adhesion of the cells and their distribution was studied via environmental scanning electron microscopy (ESEM). Specimens were fixed in 2.5% gluteraldehyde (~~Merck~~ MERCK, Germany) for at least 4 hrs at 4°C They were then dehydrated in a graded ethanol series of 30%, 50%, 90% and 100% for 5 mins at each grade, dried, and examined with a Jeol JSM-5800LV SEM at 15 kV.

Page 58, last paragraph, continuing onto page 59:

Cell/scaffold constructs were prepared for confocal laser microscopy (CLM) by staining viable cells green with the fluorescent dye Fluorescein Diacetate (FDA, ~~Molecular Probes~~ MOLECULAR PROBES Inc., Oregon). The 3D cultures were incubated at 37°C with 2 µg/ml FDA in PBS for 15 minutes. After rinsing twice in PBS, each sample was then placed in 1 mg/ml Propidium Iodide solution (PI, ~~Molecular Probes~~ MOLECULAR PROBES, Oregon) for 2 mins at room temperature to stain dead cells red. The samples were then rinsed twice in PBS and viewed under a Confocal Laser Microscope (~~Olympus~~ OLYMPUS IX70-HLSH100. ~~Fluoview~~

FLUOVIEW). Depth projection images were constructed from up to 25 horizontal image sections through the cultures.

Page 59, full paragraph:

Samples were first fixed in 3.7% formaldehyde, at room temperature, for 30 mins. After rinsing 2 times with PBS at 5 mins each time, 200 µg/ml RNAse A was added and left for 30 mins at room temperature. Phalloidin (A12379 Alexa Fluo 488 phalloidin, ~~Molecular Probes~~ MOLECULAR PROBES Inc., Oregon) was then added at in a 1:200 dilution for 45 mins at room temperature and in darkness. Samples were subsequently counterstained with 5 µg/ml Propidium Iodide solution, dried and mounted for viewing under fluorescence microscope.